Abstract

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Abstract: *DESCRIPTION* (provided by applicant): The rapid development of HIV-1 resistance to antiretroviral agents is a major clinical problem. This troubling phenomenon has been observed with each class of current clinically used anti-HIV agents. An increasingly serious clinical problem is the emergence of multidrug resistant variants of HIV-1 that show varying levels of resistance to many of the experimental drugs in the preclinical pipeline, since these analogs are directed at the same viral target as those agents to which the virus has developed resistance. There is therefore an urgent need to identify and validate new viral targets for drug discovery. HIV-1 reverse transcriptase associated ribonuclease H (RT-RNH) is one such target. Very few inhibitors of RT-RNH have been described, and those identified to date lack the drug like characteristics needed for therapeutic utility. One of the problems with RT-RNH as a target for drug discovery has been the lack of a suitable high-throughput screening (HTS) assay. We have developed a fluorescence resonance energy transfer assay readily applicable to 96-well and 384-well microplate formats with robotic manipulation to enable high-throughput screening for inhibitors of HIV-1 RT-RNH. The substrate is an 18 nucleotide 3'-fluorescein labeled RNA annealed to a complementary 18 nucleotide 5'-DABCYL modified DNA. The intact duplex has an extremely low background fluorescent signal and provides up to 50-fold fluorescent signal enhancement following hydrolysis. The size and sequence of the duplex are such that RT-RNH cuts the RNA strand four nucleotides from the 3'-end. The labeled tetraribonucleotide readily dissociates from the complementary DNA at ambient temperature with immediate generation of a fluorescent signal. The assay is rapid, inexpensive and robust, providing Z' factors of 0.8 and coefficients of variation of about 5%. The assay requires only two addition steps with no washing and is thus suitable for robotic operation. Several chemical libraries totaling more than 106,000 compounds were screened with this assay in approximately one month. Hit rates average between 0.1 - 1%. We hope that by using our HTS assay in the MLSCN suitable drug like leads may be identified and optimized.

Thesaurus Terms:
High throughput screening, HIV-1, RT-RNH, fluorescence resonance energy transfer assay, DABCYL modified DNA, tetraribonucleotide, MLSCN

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